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THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

THE DIRECTOR'S TWENTY-THIRD ANNUAL REPORT 1941-42

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Historical Preface

The Walter and Eliza Hall Institute of Research in Pathology and Medicine was founded in 1916. The Trustees of the late Walter and Eliza Hall completed the Pathological Block of the present Royal Melbourne Hospital, and provided an annual payment of £2,500 towards the upkeep of the Institute. In 1925 this was increased to £3,100, and since 1930 has been £3,200 annually. In addition, the Institute has received, since its inception, £500 per annum from the University, the Medical Staff of the Melbourne Hospital having agreed that this portion of its clinical fund should be so used. Since 1925 a further sum of £250 per annum has been provided by the University from interest on the Appeal Fund (1924), together with an annual contribution from the Clinical Research Fund.

In 1926 the late Sir Aaron Danks, then President of the Melbourne Hospital, established an Endowment Fund to support a Biochemical Department. This fund has now reached the total of £9,088. The Edward Wilson Trustees maintained this new department by contributing £1,500 per annum in 1926, 1927 and 1928, and £1,000 in 1929. This Trust also, in 1926, contributed £3,000 for the foundation of an Institute Library, and of this sum £2,000 has been invested as endowment.

The Institute has been supported by annual contributions from the Felton Bequest Committee (since 1928), the Trustees of the late Anthony Mackie (since 1933), and the Trustees of the late T. J. Sumner (since 1929). The Trustees of the late Alfred Edments have also helped us since 1937.

The Institute has received substantial support from the Department of Health of the Commonwealth Government; in the years 1927, 1928, 1929, 1930 and 1931 a total of £10,650 was received for special researches. In 1934 the Commonwealth Government entered into an agreement with the Rockefeller Foundation that each would contribute to the Institute the sum of £1,000 per annum for the ensuing three years to support work on Virus diseases, particularly those affecting the central nervous system. In 1937 the Rockefeller Foundation and the Department of Health of the Commonwealth Government agreed to continue their support of this work for a further two years. The National Health and Medical Research Council have provided additional funds during 1938, 1939, 1940, 1941, and 1942, and Mr. E. Alex. Cato has generously arranged to contribute

£1,000 per annum for the five year period from 1st January, 1939, to 31st December, 1944.

In 1932, by the will of the late Mrs. L. E. W. Carty, of Brisbane Hill, Hamilton, the Elsie Marion Carty Fund was founded. Mrs. Carty willed to the Institute a portion of her estate and a residuary interest therein to establish this Fund. It was her wish that the income should be used primarily for the assistance of promising research workers.

From 1934 to 1937 Mr. A. M. Nicholas gave an annual sum of £250 towards the salary of the Assistant Director, and Mrs. Nicholas generously continued this contribution in 1938 and 1939.

In 1936 the Carnegie Corporation of New York paid the travelling expenses of Dr. W. Feldberg and his family to Australia, and contributed in addition 4,500 dollars, which, with £1,100 collected by Mr. Reuben Hallenstein, made it possible to maintain this worker for two years.

The establishment of the National Health and Medical Research Council in 1937 has aided greatly in the development of this and other institutes for medical research in Australia.

It was finally decided in May, 1938, to rebuild the Royal Melbourne Hospital on the new site in Parkville. The Committee of Management subsequently accepted the responsibility for a new Institute building, the cost of which was estimated at £45,000. Towards this amount Mr. G. R. Nicholas and the family of the late Mr. A. M. Nicholas—Mrs. A. M. Nicholas, Mr. Maurice Nicholas, and Mrs. William Gluth—contributed £20,000. Mr. Russell Grimwade and the Broken Hill Proprietary Company Limited gave each £1,000, and a few other gifts have brought the special fund for this purpose to a total of £22,075.

In 1942 the Gordon Clunes Mathison Research Scholarship was founded by a bequest of £6,500 from the estate of his mother, Mary Martin Mathison, and the sum of £476 from the estate of Mr. Joseph Smith was added to the Biochemical Endowment Fund.

The Institute is controlled by a Board consisting of representatives of the Walter and Eliza Hall Trust, of the University of Melbourne, and of the Committee of Management and Medical Staff of the Royal Melbourne Hospital. During the Great War Professor Sir Harry Allen acted as Honorary Director. In 1919 Dr. S. W. Patterson was appointed first Director; in April, 1923, he resigned, and was succeeded by Dr. C. H. Kellaway in August, 1923. During the interval Mr. H. Dew acted as Director.

The Director's Twenty-Third Annual Report

TO THE BOARD

OF THE

Walter and Eliza Hall Institute of Research in Pathology and Medicine

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JULY, 1942.

Almost the whole of the research of the Institute is now directed towards the war effort. Dr. Burnet has continued his studies on immunization against influenza, and is engaged, with the assistance of Captain Ferris, A.A.M.C., and Dr. Stephen Williams, of the Commonwealth Serum Laboratories, on large scale immunization in the army in Victoria. Mr. Rawlinson has developed an excellent method of testing for arsenic in biological fluids, and Dr. Gottschalk's work on fermentation should find application in the manufacture of power alcohol. Dr. Reid has completed, in my laboratory, a study of the pharmacologically active substances in human serum. Work has also been in progress on the concentration and chemical drying of serum and upon methods of preparation of pyrogen-free water. A full account of these activities is given later in this report.

During the year 72,000 members of the Services have been blood grouped, and in addition blood grouping and blood tests have been carried out on a large number of emergency donors for the Red Cross Transfusion Service. An active centre for the cleaning and sterilization of glassware for the Red Cross Emergency Transfusion Service has been set up in the old Institute building, where also Miss Bick is engaged in the processing of human serum for civil emergency. These developments have necessitated the occupation of more laboratories in the new Institute building, and the virus department was installed there in April of this year.

The occupation of the new Royal Melbourne Hospital by the 4th U.S. Base Hospital has provided a very close and pleasant association with the staff of that unit, and several officers from the hospital pathological service have been temporarily accommodated in Institute laboratories.

The special war research fund, contributed by Mr. A. P. Adams, of Gilgai, Nagambie, has made it possible to obtain from the Division of Animal Health of the Council for Scientific and Industrial Research the services of Dr. Beveridge to aid Dr. Burnet in the work on influenza immunization.

In September of last year, in response to a request by the Scientific Advisory Committee of Great Britain, I was sent, under the aegis of the National Health and Medical Research Council, to the United States, Canada and United Kingdom. I left Australia by air on 29th September, and returned by sea, reaching Melbourne on 17th April, 1942. I spent nearly two months in the United States and Canada, and nearly three months in Great Britain, seeing the recent advances in war medicine and arranging for a better liaison with medical research in this country. Except for this period of absence, I have remained a member of the Army Medical Directorate.

Mr. Arthur Hughes, who for some years has combined the duties of electrocardiographer for the Hospital with those of looking after the mechanical equipment of the Institute and purchasing our materials and apparatus, has left us for the duration of the war to take a post in the Department of Munitions.

This year three of the new series of Institute monographs have appeared. The first, by Dr. F. M. Burnet, Miss Mavis Freeman, Dr. A. V. Jackson and Miss Dora Lush, on the production of antibodies; the second by Dr. E. Graeme Robertson, on encephalography, and the third by Dr. F. M. Burnet and Miss Clark on influenza.

It is a great pleasure to offer our congratulations to the assistant director of the Institute, Dr. F. M. Burnet, and also to Col. N. Hamilton Fairley, C.B.E., who was assistant director of the Institute in 1920-21, and was again a member of its staff in 1927-1928, on their election as fellows of the Royal Society.

This year a bequest of £6,500 from the estate of the late Mrs. Mathison has provided for the foundation of a Gordon Clunes Mathison Research Scholarship in memory of her son, Dr. C. G. M. Mathison, a distinguished young physiologist, who was the first director of the Clinical Laboratories at the Royal Melbourne Hospital before the foundation of the Hall Institute. Dr. Mathison died of wounds inflicted at Gallipoli on 18th May, 1915.

VIRUS DEPARTMENT.

The work of the Virus Department has been centred on the problem of immunization against virus influenza, and, as in the previous year, extensive experiments have been carried out on human volunteers. During the latter half of 1941 the preliminary account of Hirst's method of titrating the virus became available in Australia. This method provides a revolutionary simplification of the standard procedures in influenza virus research, and it has already been extensively applied in this laboratory. In addition to its use in influenza virus research, the Hirst test, with certain modifications, is also applicable to Newcastle disease virus and vaccinia virus. Both these viruses have been studied from this point of view in the year under review.

Influenza.

No epidemic of virus influenza was recognized during 1941. In December there was a well marked epidemic of clinical influenza, but investigations on what appeared to be a typical sample of cases in the Royal Melbourne Hospital nursing staff gave no evidence that either A or B type virus was responsible.

The study of attenuated influenza virus strains as immunizing agents was extended this year to type B virus. The strain B (Lee) was taken through 40 amniotic passages and then used for immunization. The virus in the form of allantoic fluid was sprayed into the nose and throat of a group of 22 student volunteers. No significant symptoms resulted, and a sharp antibody response was induced in nine of the ten persons whose initial antibody was below a certain level. The rise was evident both by the Hirst tests and by the conventional mouse lung titrations.

With the results of this experiment and of the past two years' work with attenuated A type strains as a basis, a larger scale experimental immunization with pooled A and B viruses was carried out. Through the co-operation of D.D.M.S. Vic. L. of C. (Col. Gordon Shaw), 120 volunteers were inoculated with the mixed virus vaccine. This consisted of pooled allantoic fluids from eggs infected respectively with the A strains W.S. and Melbourne after 100 and 56 amniotic passages, and the B strain Lee after 40 amniotic passages.

Coryzal symptoms were reported by slightly over one-fourth of the men, but many of these were probably unrelated to the inoculation. The symptoms were trivial, and usually consisted of a mild coryza with some sneezing about 48 hours after the inoculation. None of the men were off duty as a result of their symptoms.

A comparison of antibody levels at the time of inoculation and three weeks later was made by Hirst's method. Significant antibody rises against the three strains were observed as follows: Melbourne, 28%; W.S., 31%; B, 20%. There was a partial correlation between Melbourne and W.S. rises, but, as would be expected, the B rises were quite independent.

The most hopeful feature of these results was that the antibody rises were concentrated amongst the subjects with low initial antibody. Although there is only a partial correlation of susceptibility to influenza with low antibody level, this suggests that the method should automatically produce the greatest degree of immunity in those most in need of it. Whether this immunity is sufficient to protect against the natural disease remains to be determined.

Incidentally to this work much effort has been directed towards improving the methods of obtaining, storing and administering the virus. Eggs are now inoculated by the suballantoic method and opened after being chilled for two hours to prevent bleeding while the allantoic fluid is removed. The fluid is lightly buffered and stored in the refrigerator. Strains W.S. and B keep up to 10% of the initial titre for a month or more, but "Melbourne" falls much more rapidly.

Influenza in Monkeys.

The work on cynomolgus monkeys given high titre virus intratracheally that was reported in the last Annual Report has been completed as far as the limited number of monkeys would allow. A small experiment on immunization showed the effectiveness of intratracheal or subcutaneous inoculation of living virus and the relative inadequacy of living virus intranasally or of formalin killed virus subcutaneously.

Influenza B in Australia.

No current outbreaks of influenza B have been observed, but a survey of sera taken at approximately yearly intervals from members of the laboratory staff has shown that one such infection occurred in 1938-39. In all probability a fairly acute illness in November, 1938, represented the B type infection.

As others have shown, influenza virus B grows readily by the methods of amniotic or suballantoic inoculation and a passage series of 100 generations of amniotic transfer has been completed. No obvious changes in pathogenicity for mice or chick embryos were induced.

The Recent History of Influenza.

The mass movements of armies or refugee populations in war-time provide conditions under which respiratory infections flourish, and the possibility of a pandemic appearance of influenza like that of 1918 must be kept in mind. Dr. Burnet and Miss Clark have prepared a study of influenza since the appearance of the 1889 pandemic in the light of modern knowledge of the virus. The monograph contains a review of experimental work to the end of 1941, but its main object is to discuss what could or should be done in the event of a pandemic comparable to that of 1918. The greater part of the text is devoted to a discussion of various aspects of the great pandemic. The work has been published as Monograph No. 4 of the Institute Series.

General Studies with Hirst's Phenomenon.

As the information available was limited to Hirst's preliminary note, a fairly comprehensive superficial survey of the phenomenon was made to serve as a background until detailed reports from Hirst and his collaborators were received. An interesting feature not so far noted elsewhere is the wide range of red cells that are agglutinable by influenza virus preparations. They include frog and tortoise, several birds, and a curiously random group of mammalian blood cells: human, guinea-pig, mouse and marsupial mouse being susceptible; monkey, rabbit, horse, cow, sheep and ferret are either wholly unagglutinated or very weakly affected.

A number of viruses other than influenza were tested, but only Newcastle disease and vaccinia viruses have so far proved active.

Limited studies on the nature of the agglutinin suggest that it is in part associated with virus particles, in part free in solution. Seitz filtrates, for instance, contain proportionately more agglutinin than virus, although both agents are greatly reduced.

Virus preparations completely inactivated with formalin retain their agglutinating power unreduced for some time.

An examination of the immune serum-virus interaction by means of the Hirst test has provided an interesting and complete confirmation of the principles deduced by Burnet, Keogh and Lush in 1936 for the virus-antibody reaction in general, viz.:

(1) The percentage law of Andrewes and Elford.

(2) The C.P. = constant rule.

(3) The action is completely reversible, and the time taken to reach equilibrium level is demonstrable only when dilute reagents are used.

The agglutinin-red cell union is readily dissociable by the addition of immune serum, and in the case of Newcastle disease virus by simple washing. After dissociation of the virus, however, the cells have changed their character in so far as they are no longer agglutinable by Newcastle disease virus, but still agglutinate with all influenza viruses.

Newcastle Disease Virus.

Dr. Burnet has made a fresh study of this virus in the chick embryo under the stimulus of the discovery that it produces active Hirst agglutination.

There are striking resemblances in its behaviour to that of fully adapted influenza virus strains, and it is very near the truth to say that if attention is confined to the chick embryo the only possible way of differentiating N.D.V. from the adapted influenza viruses is by immunological tests. The viruses have the same particle size, produce the same characteristic haemorrhagic encephalitis, induce lung lesions in the embryo in a proportion of eggs inoculated amniotically, and multiply freely when introduced into the allantoic cavity. The allantoic fluids in both cases have high Hirst agglutinin and virus content.

N.D.V. in the form of high titre allantoic fluid will induce subclinical infection in ferrets and pulmonary consolidation of influenzal type in mice.

For these reasons Dr. Burnet has claimed that N.D.V. represents a virus having a common ancestor with the human strains of influenza virus.

Vaccinia Virus.

Since October Dr. Nagler has been working with a reaction produced by vaccinia virus, which is analogous to the Hirst phenomenon. The activity of vaccinia virus is, however, much more restricted than that of the influenza or Newcastle disease viruses. Fowl cells only are agglutinated, and amongst fowls only about 50% are suitable. Dr. Nagler and Miss Clark found that this difference between fowls was unrelated to sex, age or breed. So far the only satisfactory virus preparations have been made from lesions on the chorioallantois; raw calf lymph has shown no activity. With suitable reagents it can be shown that the agglutination is specifically neutralized by immune serum, and the method can therefore be applied to the titration of antibody in human sera.

Serological Classification of Invertebrates.

Miss Clarke has completed the study of the serological reactions of crustacean sera to which reference was made in the last report. The most interesting feature which emerged with a wider range of crustacea was a division into groups which showed no mutual cross precipitation whatever. These groups corresponded sometimes with the families of the systematic classification, but in some cases a number of families were included in one of these major serological groups. Experiments in collaboration with Mr. Rawlinson showed that the copper-containing protein haemocyanin was the only important antigen involved in these precipitation tests. A number of interesting problems emerged from this work, but as the whole investigation was of necessity done at irregular times which could be spared from work of more practical importance, it has been impossible to follow them up.

F. M. Burnet:

- ''Influenza A Infections of Cynomolgous Monkeys,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1941, 19, 281.
- ''Growth of Influenza Virus in the Allantoic Cavity of the Chick Embryo,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1941, 19, 291.
- ''Charles Mackay Lecture. The Biological Approach to Infectious Disease,'' ''Medical Journal of Australia,'' 1941, 2, 607.
- ''The Ecology of Syphilis,'' ''Bulletin of Genitoinfectious Diseases,'' 1941, 4, No. 8.
- ''The Affinity of Newcastle Disease Virus to the Influenza Virus Group,'' ''Australian Journal of Experimental Biology and Medical Science'' (in the press).

F. M. Burnet, M. Freeman, A. V. Jackson, and D. Lush:

- ''The Production of Antibodies. A Review and a Theoretical Discussion.'' Monographs from the Walter and Eliza Hall Institute of Research in Pathology and Medicine, Melbourne, No. 1, 1941.

F. M. Burnet and Ellen Clark:

- ''Influenza.'' Monographs from the Walter and Eliza Hall Institute of Research in Pathology and Medicine, Melbourne, No. 4, 1942.

F. M. Burnet and M. Foley:

- ''Complement Fixation in Influenza with Embryo Fluid as Antigen,'' ''Medical Journal of Australia,'' 1941, 2, 468.

F. M. Burnet and D. D. Faris:

- ''The Technique of Quantitative Chorioallantoic Virus Titration.'' Submitted to the ''Journal of Immunology.''

F. P. O. Nagler:

- ''Application of Hirst's Phenomenon to the Titration of Vaccinia Virus and Vaccinia Immune Serum,'' ''Medical Journal of Australia,'' 1942, 1, 281.

DEPARTMENT OF PHYSIOLOGY.

The work of this department was carried on during my absence abroad until early in 1942 by Dr. George Reid, Junior Research Fellow of the National Health and Medical Research Council. The equipment was then moved to the new building, but the laboratories have not yet been reopened since Dr. Reid, after a brief period as lecturer in Histology in the Anatomy Department of the University, has enlisted for service in the army, as has also my senior laboratory assistant, Mr. Peter Silberstein.

During the period under review Dr. Reid continued the study of the pharmacologically active substances which are absent from blood, but which appear in serum when blood clots. Three types of activity have been investigated: (1) The vaso-constrictor and smooth muscle stimulating property of serum, (2) the phenomenon described by Brodie in 1900—a fall in the systemic blood pressure of the cat immediately following the intravenous injection of serum, and (3) the alleged presence of adenyl compounds in serum.

Using the isolated strip of ox carotid, the isolated guinea-pig's jejunum, the isolated rabbit's ear and the uterus of the virgin rat as test objects, it was shown that the vasoconstrictor and muscle stimulating substance of human serum is derived from the blood platelets, in which it exists preformed, and from which it can be liberated by extraction with saline. Of various sera tested, that prepared from centrifuged plasma has minimal and that prepared from whole blood has maximal activity; serum prepared from plasma separated by means of a cream separator is somewhat more active than that prepared from centrifuged plasma. Serum protein prepared by the method of Hardy and Gardiner is inactive. The vasoconstrictor substance is not extractable from serum by ether, methyl alcohol or ethyl alcohol. It can withstand a temperature of 100° C., is dialysable through cellophane membranes, and can be obtained in an ultrafiltrate. Reports on the use of human serum for transfusion in man yield no evidence that vasoconstrictor or muscle stimulating substances have been responsible for reactions, but our results indicate that simple methods are available, and are at present in use, to prepare relatively inactive serum.

The fall in systemic blood pressure in the cat following the intravenous injection of serum was shown to occur after division of the vagus and in decapitate preparations in which the spinal cord was destroyed. It is associated with pulmonary vasoconstriction indicated by a steep fall in the pressure in the pulmonary vein and a sharp rise in the pressure in the pulmonary artery. The phenomenon depends on the presence of a heat-labile dialysable substance liberated from the platelets in the process of clotting, and this appears to be responsible for all the activity of homologous serum. The effect can also be produced, as Brodie showed, by foreign proteins. It cannot be produced in the rabbit or dog, nor is there any evidence that it occurs in man.

No active adenyl compounds could be demonstrated in human serum, but an adenosine-inactivating enzyme was shown to be present.

As a result of this work, it was decided to investigate the possibility that a vasoconstrictor substance derived from the blood platelets might play a part in the mechanism of haemostasis. Preliminary experiments have shown that three patients with prolonged bleeding times and reduced platelet counts yielded sera with very little vasoconstrictor activity. One patient with hereditary thrombasthenia whose bleeding time was very prolonged, but whose platelet count was normal, yielded an actively vasoconstrictor serum. In these experiments a dried serum prepared from whole blood and reconstituted before testing was used as a standard. Because of the possible part that the capillaries themselves may play in the mechanism of haemostasis, it is proposed to investigate the reactions to vasoconstrictor substances of the skin capillaries of normal and purpuric individuals.

Experiments on Skeletal Muscle:

The alteration in the adenyl content of the skeletal muscle of the rat after denervation was also investigated. It was found that after denervation active adenyl compounds were lost from the muscle at the same rate as the muscle tissue atrophied. The time relationships of the appearance of spontaneous fibrillation, of failure to obtain fascicular twitching by physostigmine and of change in sensitivity to acetylcholine in the denervated muscle of the cat were also studied. As is well known, fasciculation is unobtainable at an early stage of nerve degeneration (approximately two days), and fibrillation appears after five days. Our experiments showed that hyper-sensitivity to acetylcholine is established after five days, although contractural responses may be seen as early as the third day. Fibrillation is probably not attributable to the action of acetylcholine on highly sensitized muscle because doses of curare, which considerably reduce the response of denervated muscle to acetylcholine, have little or no effect on fibrillation in the same muscle. Because the fasciculation produced by physostigmine depends on an intact peripheral portion of motor nerve, an attempt was made to "remove" the nerve endings in a muscle by intra-arterially injected novocain. This led to no definite conclusion concerning the site of action of physostigmine because a further curare-like action of novocain was observed—it abolished fasciculation in doses which had little or no effect on neuromuscular transmission. In addition to this and other known curare-like actions of novocain, the drug, in larger doses, was shown to have a direct effect on the muscle itself, reducing the response to direct stimulation, reducing the twitch-tension ratio, causing failure of maintenance of tetanus and temporarily abolishing fibrillation in denervated muscle.

C. H. Kellaway, G. Reid, and E. R. Trethewie:

"Circulatory and Other Effects of the Toxin of Cl. Septique," "The Australian Journal of Experimental Biology and Medical Science," 1941, 19, 297.

G. Reid and Marjorie Bick:

"Pharmacologically Active Substances in Serum," "The Australian Journal of Experimental Biology and Medical Science," 1942, 20, 33.

"Some Pharmocological Properties of Serum with Special Reference to its Use as a Blood Substitute," "The Medical Journal of Australia," 1942, 1, 245.

G. Reid:

"The Change in the Adenyl Content of Skeletal Muscle After Denervation" (in the press).

"Some Observations on Fasciculation and Fibrillation in Skeletal Muscle, with Special Reference to the Action of Novocain on the Neuromuscular Mechanism" (in the press).

E. R. Trethewie and Eleanor Williams:

"The Use of Kangaroo Tendon for Muscle and Tendon Suture," "The Australian and New Zealand Journal of Surgery," 1942, 11, 207.

J. D. Hicks, E. R. Trethewie, and Eleanor Williams:

"Experimental Studies in the Use of Amnioplastin," "The Australian and New Zealand Journal of Surgery," 1942, 11, 268.

BIOCHEMICAL DEPARTMENT.

The department was moved into the new Institute early in May, 1941, to provide space for the blood transfusion centre in the old Institute. Owing to the incomplete state of the new building, no delicate apparatus could be unpacked till the middle of August. Much of the work incidental to such a move, such as overhauling and modifying apparatus to fit the new building, has had to be done by the research staff, and is being completed as time and opportunity offer.

Mr. Rawlinson continued his experiments on the distillation of arsenious chloride and its accumulation in sodium hydroxide solution. He found, however, that the arsenic present in all the pyrex glass available here was sufficient to render uncertain the methods based on the use of sodium hydroxide as a reagent for the trapping of arsenic. It is understood that arsenic-free glass is now available in some countries. He has spent the rest of the year in a study of the electrolytic method of the generation of arsine from solutions of arsenates and arsenites. Although this method was first proposed many years ago, it has been repeatedly condemned as unreliable. He has traced the errors to the liability of the junction between the platinum lead-in wire and the mercury cathode to become wet with acid, thus converting the mercury to an intermediate electrode of small reducing power. He has set up a simple, cheap and rugged apparatus which gives rapid and reliable results with only a few micrograms of arsenic, reduces arsenates as well as arsenites, and eliminates the costly and scarce arsenic-free zinc and most of the arsenic-free sulphuric acid required for the standard methods.

Dr. Gottschalk has completed the investigations concerning the relationship between alcholic fermentation and polysaccharide synthesis by living yeast with glucose, fructose and mannose as substrates. In collaboration with Mr. Rawlinson, an apparatus has been developed which permits the measurement of fermentation and respiration with amounts of yeast suspensions large enough to study the total balance of the substrates added. With this apparatus Dr. Gottschalk has studied the mechanism of the "phytochemical reductions" by fermenting yeast cells under aerobic and anaerobic conditions. Acetaldehyde added to compressed yeast respiring and fermenting in glucose phosphate solution displaces completely that part of the oxygen consumed which in normal respiration (with glucose as substrate) reoxidizes dihydrocozymase. By displacing molecular oxygen as hydrogen acceptor for reduced cozymase, the acetaldehyde is phytochemically reduced, and that in an amount explainable only if per mol triosephosphate completely oxidized 2 mol dihydrocozymase are intermediately produced. A quantitative study has been made of the two steps involved in the phytochemical reduction of diacetyl by fermenting yeast under anaerobic conditions (diacetyl acetyl methyl carbinol 2, 3-butanediol). The fermentation acetaldehyde, by these reductions deprived of its dihydrocozymase, is dismuted to acetic acid and alcohol. The rate of reduction of acetyl methyl carbinol to 2, 3-butanediol is twice the rate of reduction of diacetyl to acetyl methyl carbinol. $\frac{m}{80}$ acetaldehyde, added to fermenting baker's yeast in nitrogen, inhibits reversibly the rate of fermentation.

Until my departure from Australia, Mr. Holden continued work on the drying of sera for the investigation of their pharmacologically active constituents. He has also been engaged in a study of the methods of preparation of glycollic aldehyde, a substance desired for research on carbohydrate metabolism. Of recent months the increasing scarcity of materials both for research and for many urgent purposes has forced him to devote much of his time to advising and helping others in their search for suitable substitutes. The gradual moving of the equipment of other departments has also consumed much time owing to the extreme shortage of men who are familiar with the erection and maintenance of elaborate scientific apparatus.

W. A. RAWLINSON:

''The Effect of Oxidizing Agents on Haemocyanine,'' ''The Australian Journal of Experimental Biology and Medical Science,'' 1941, 19, 137.

''The Determination of Minute Amounts of Arsenic in Biological Materials: A New Type of Electrolytic Cell for the Production of Arsine'' (in the press).

A. GOTTSCHALK:

''The Chemical Equations of the Phytochemical Reductions of Fermenting Yeast Cells and their Relationship to the Pasteur Effect'' (in the press).

CLINICAL RESEARCH.

Purification of Water.

The experimental work on the removal of pyrogens from water by treatment with charcoal has been continued. It was found that if tap water or fresh river water was shaken for 30 minutes with four parts per thousand of charcoal, filtered through filter paper, made up into saline and autoclaved, this could be given intravenously to rabbits without any rise in temperature. Saline made from fresh river water treated in this manner was also given to a number of patients without producing any pyrogenic reactions. Dr. Henderson and Miss Bick are now investigating a number of samples of locally prepared charcoal. Though these are not so effective in removing pyrogens as the imported charcoal used in the original experiments, satisfactory results can be obtained with some samples by increasing the amounts used.

Serum Preparation.

The use of a modified milk separator for the separation of human plasma was investigated. There was no haemolysis when oxalated bloods of different groups were passed in succession through the separator. Satisfactory separation without haemolysis could be obtained from blood stored for thirteen hours after collection, but not if a longer period of time had elapsed before separation. The average yield of plasma was 55% if the blood was fed into the separator at four litres per hour. After separation the plasma was clotted by the addition of calcium chloride and filtered through Seitz bacterial filters. This method is being used extensively for the large scale preparation of human serum.

Concentration and Drying of Serum.

The method of precipitating proteins with alcohol and ether described by Hardy and Gardiner has been applied to drying serum for intravenous use. The serum to be dried is first concentrated to one half or one third of its volume in cellophane tubes placed in a draught of dry warm air. The concentrated serum is then added to ten volumes of a mixture of seven parts ethyl alcohol and three parts of anhydrous ether cooled to-12° to-14° C. The precipitate, after standing three hours in the cold, is washed aseptically with ten volumes of ether and dried *in vacuo.* The dried precipitate is a fine white powder which goes into solution very rapidly, giving a perfectly clear reconstituted serum. This has been administered to a number of patients without reaction and with beneficial results. The possibility of expanding this method is being investigated, and it is hoped that the expense of using large volumes of organic solvents can be overcome by distillation and recovery.

Liver Function Tests.

In an attempt to assess the value of the hippuric acid test for liver function a review was undertaken of tests performed by Miss Splatt during the past three years in the biochemical department of the Royal Melbourne Hospital. A number of new cases was investigated, some of them by serial tests. These included 50 patients with thyroid disease. It was concluded that the test was a perfectly safe and reasonably delicate measure of liver function. Certain of the cases of thyroid disease showed a significant decrease in liver function. The Takata-Ara reaction was similarly investigated. It appears to be of little value as a test of function, but is a possible aid to the differential diagnosis of parenchymatous and obstructive jaundice.

H. B. Rudduck, Marjorie Bick, and Margaret Henderson: "A Note on the Use of the De Laval Separator in the Preparation of Human Serum," "Medical Journal of Australia," 1941, 2, 416.

Margaret Henderson and Beryl Splatt: "The Value of the Hippuric Acid Test and Takata-Ara Reaction in the Investigation of Hepatic Deficiency," "Medical Journal of Australia," 1942, 1, 185.

NEUROLOGICAL RESEARCH.

Dr. E. Graeme Robertson's monograph on Encephaolography has been completed and published. In it he has described and fully illustrated a special technique for conducting this investigation. The essential feature of this is the observation by radiography of the passage of a limited quantity of air from the spinal canal to the highest point to which it can rise. The course followed by the injected gas is described and the factors which determine this are analyzed. Dr. Robertson shows how it is possible by consideration of those factors to direct the gas towards any of the intracranial spaces. Either the ventricles alone or selected parts of the subarachnoid space can be filled. The method allows of satisfactory visualization of the fourth ventricle and the Sylvian aqueduct, and thus is useful in the study of lesions in the posterior cranial fossa. Both the author and the editor are to be congratulated upon a clearly expressed and beautifully illustrated work.

Dr. Robertson has continued to examine specimens of neurological interest kindly provided by Dr. Wright-Smith. A number of interesting cases have been encountered in which the results have been correlated with clinical findings. This material has been used for clinico-pathological demonstrations to students.

BLOOD BANK.

During the past twelve months 1,581 donors of the Red Cross Voluntary Transfusion Service have been bled to supply blood and serum for the bank. The average weekly supply of blood for emergency transfusions is thirteen pints, and the blood collected from 909 donors has been used for serum production for civilian needs. This work has been carried out entirely upon a voluntary basis, and we offer our grateful thanks to Dr. Ella MacKnight and Dr. Dorothy Synan, honorary medical officers to the blood bank, and to Dr. Alice Correll, Dr. Isabel Ireland, Dr. Marion Wanliss, Dr. Joy Bell, Dr. Rachel Taylor, Dr. Doris Officer, and Dr. John Perry, who have been responsible for bleeding the donors; to the Red Cross aids, who have been responsible for the care, assembly and sterilization of all equipment used, and finally to the donors themselves.

RED CROSS BLOOD TRANSFUSION SERVICE.

There has been great expansion in the work of this service during the past year, chiefly to provide pooled serum for the Services, and for possible civilian air-raid casualties. As a result there have been 11,397 calls for donors, as compared with 2,955 in the previous year.

As in the past, the preliminary examination and collection of blood samples from prospective donors have been carried out voluntarily by medical women, assisted by science graduates, Red Cross aids, and members of the Institute staff, several of whom have continued to give voluntary help in the evenings. This year Dr. Rachel Gross has given much valuable help by visiting factories and other large institutions, whose executives have co-operated in allowing their employees to be examined during working hours.

The laboratory work involved in testing donors has, as before, been carried out almost entirely at the Institute. During the twelve months ended 24/4/42, blood group (and sub-group tests when necessary) and Kahn tests, serum titre and haemoglobin estimations have been performed by Dr. Jakobowicz and Dr. Karoly on blood samples from 9,718 new donors, together with 283 serum titre estimations and 327 Kahn tests on donors enrolled previously. The haemoglobin content of the blood of a number of donors who have given blood repeatedly has also been examined, involving 3,330 tests. In a small percentage of these the original haemoglobin level has not been maintained. The individuals concerned have been notified, and given the choice of treatment by their own doctor or by Dr. Bryce on behalf of the Red Cross Service. All of those treated by Dr. Bryce have shown satisfactory response to adequate iron therapy.

A notable expansion of the work of the service has been the training of Red Cross Aids, including the four from Tasmania, under the capable direction of Miss Bick. Thirty-five of these voluntary workers have been enrolled, and the majority, without any previous laboratory experience, have become proficient in the technical procedures necessary for the assembling and sterilizing of equipment, and in assisting at the bleeding of donors.

Immediately after the declaration of war by Japan, the Service was "mobilized," according to the plans outlined some months previously, so that supplies of fresh blood could be made quickly available if civilian air-raid casualties occurred here. Four blood collecting centres were set up in different parts of Melbourne. These will be staffed by voluntary medical officers who have gained their experience at the Blood Bank, Red Cross Aids trained by Miss Bick, Red Cross clerks and transport drivers. Their equipment has been prepared at the "Centre" at this Institute. Several trials to test the efficiency of the arrangements have been made, both by day and night, two of them in conjunction with official air-raid tests.

Early this year it was decided that the rate of production of pooled serum in Melbourne should be increased. The Red Cross rooms at No. 12 Collins Street were not large enough to accommodate the increased numbers of donors required, but the Committee of Management of the Royal Melbourne Hospital provided a suite of rooms on the third floor of the new Royal Melbourne Hospital. Adjacent sterilizing rooms were made available on the same floor in the new Institute.

The greater part of the serum prepared in Melbourne is processed at the Commonwealth Serum Laboratories, but Miss Bick is preparing some eight to ten litres a week in excess of normal needs. In this work, involving a great deal of evening overtime, she has had the invaluable co-operation of Miss Splatt, and of her assistant, Miss Best. Through the courtesy of the Medical Superintendent, Dr. Curtis, and other members of the staff of the Royal Melbourne Hospital, Miss Bick has been able to organize a new Collecting Centre in the emergency theatre for this work and that of the Blood Bank. Drs. MacKnight, Synan, Correll, Henderson and Ireland have continued to give voluntary service in bleeding donors, and more recently Drs. Doris Officer, Rachel Taylor, Marion Wanliss, Joy Bell and John Perry have also given their services. Training in transfusion with standard "Soluvac" apparatus has been given to teams from 115 General Hospital. Drs. Jean Gunson and Campbell Duncan, from Tasmania, and Miss Lyons, who is in charge of equipment for the Blood Bank recently established at the Alfred Hospital, also spent some time at the Institute. Red Cross Aids, trained by Miss Bick, have been assisting at the Alfred Hospital Blood Bank.

Dr. Jakobowicz has given valuable help to Miss Webster, of the Donor Call Office, in the selection of suitable groups of donors for pooled serum.

Some experimental work is in progress, on apparent seasonal variation in iso-agglutinin titre, and on persistence of human iso-agglutinins in rabbits after transfusion with wet and dried (Hardyized) serum of rabbits previously immunized with human red cells.

LUCY M. BRYCE and RACHEL JAKOBOWICZ:

> "The Interaction Between Anti-A Agglutinins in Group B Serum and Receptor Substances in Group A Serum," "Medical Journal of Australia," 1941, 2, 451.

WAR WORK.

A large amount of work of a routine nature has been carried out this year at the Institute. Nearly 72,000 blood typing tests have been carried out for the Services, and in this work we have had the voluntary help of Dr. Rachael Gross, Dr. Margaret Ashton, Mrs. Hughes, Mrs. Alan Jackson, Mrs. Treloar, Mrs. Oppenheim, Miss Helen Ferguson, Miss Phyllis Rountree, Miss Mavis Freeman, Mrs. Taylor, Mrs. Newman, Mrs. Branigan, and Miss Kathleen Williams.

Most of the catgut testing for the army is now being done elsewhere, but Miss Williams has been available as a stand-by, and in that capacity has had to do nearly a thousand tests.

Miss Williams also assisted the army in a survey of the incidence of carriers of amoebic dysentery among prisoners of war and internees. This work was carried out in temporary field laboratories at camps in Northern Victoria, and involved Miss Williams's absence from the Institute for a period of six weeks, during which time some 600 persons were investigated.

In collaboration with Dr. Wheatland, of the Commonwealth Serum Laboratories, a mixed vaccine against cholera, dysentery and enteric infections was tested, eighteen volunteers from the Institute staff being injected and bled at appropriate intervals.

ROUTINE HOSPITAL SERVICES.

Bacteriology and Clinical Pathology.

The routine work in this department still continues to increase, and the difficulty of carrying it out satisfactorily has been increased by the enlistment of the senior male laboratory technician and by illness of other members of the staff. The war has thrown further burdens on this laboratory because, in addition to a number of special investigations for members of the Services, all the personnel of the Hospital (about 1000 persons, including auxiliaries and canteen workers) have been blood grouped.

Dr. Gardner has carried out for the Hospital a special investigation of all the available germicides, as a result of which

Zephiran is now being used in the wards and theatres, with considerable saving of expense. Further substantial saving of Hospital maintenance charges has been achieved by the manufacture by the staff of this laboratory of the extracts for use in the Allergic Clinic.

Some interest attaches to the investigation of recently occuring cases of cerebrospinal meningitis. The meningococci isolated in 25 cases have all proved to be type 1, and 80% of these patients have been over 40 years, some even over 60 years of age.

Dr. Gardner has continued typing all cases of pneumonia, and has investigated the bacterial flora in 53 cases of empyema during the last two years and a half. Of these thirteen only have been associated with pneumonocci. In 23 anaerobic gram negative fusiform bacilli have been present, either alone or in association with other organisms, such as streptococci. These observations stress the importance of oral hygiene not only during the course of pneumonia, but also before the administration of general anaesthetics.

BIOCHEMICAL DEPARTMENT.

In addition to the routine biochemical work of the Hospital, some special investigations have been undertaken. An investigation of liver deficiency by means of the Hippuric Acid test and Takata-Ara reaction has been carried out for the past three years, and was completed in association with Dr. Margaret Henderson.

Under the direction of Dr. L. Ivan Maxwell, the effect of Vitamin B_1 injection on the gastric reaction of patients with achlorhydria was studied.

The Exton-Rose test has been compared with the Glucose Tolerance test on a small number of patients. This test can be completed in one hour, but requires double the amount of glucose, and, in view of the shortage of both glucose and blood sugar reagents, it was decided to postpone to a more favourable time a more extensive series of tests.

Barbara Wood, Beryl Splatt, and Ivan Maxwell:

> ''The Investigation of the Effect of Administration of Vitamin B_1 Upon Gastric Secretions and the Motor Activity of the Stomach—A Preliminary Report'' (in the press).

MORBID ANATOMY.

During the year 538 autopsies were performed. Dr. C. H. Mollison and Dr. R. J. Wright-Smith were responsible for the examination of 1,736 biopsy sections from 1,348 specimens. Sections from autopsy material numbered 636. Routine examinations of the appendix removed at operation was carried out on 419 occasions. The photography continues to be done by this department.

MUSEUM.

To make way for the Blood Bank, the greater part of the museum was transferred to the new Hospital. Two hundred specimens were retained for teaching purposes, but were not available for ordinary use by honoraries and students.

It is proposed to bring the majority of specimens back and to re-establish the museum for general use. The remounting of old specimens has continued. New specimens included examples of cerebral abscess, submaxillary calculus, mucocele of appendix, sarcoma of the uterus, myoma of stomach, suppurative nephritis, transposition of viscera, aneurysm of the aorta. Specimens have been used for the teaching of students and graduates and in refresher courses for the army.

TEACHING.

Dr. Wright-Smith has given the usual course of tutorial lectures in pathology to fourth year students in December and February. Lecture demonstrations in morbid anatomy have been given once weekly to fifth and sixth year students, and lectures in elementary bacteriology have been given to the student nurses in the Hospital. During May, 1942, medical and surgical teams were given daily demonstrations on post-mortem material. Tuition in Clinical Pathology was given to fifth year medical students by Dr. H. Gardner.

LIBRARY.

Our thanks for the gifts of journals and books are due to the following:—The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; The London Hospital; The Medical Research Council; The Mary Imogene Bassett Hospital, Cooperstown, New York; The Middlesex Hospital Medical School; New York State Department of Health, Division of Laboratories and Research; Department of Pathology, University of Oxford; Rockefeller Institute, New York; Dr. R. J. Wright-Smith; The South African Institute for Medical Research; The University of Harvard, Department of Tropical Medicine; and The University of Pennsylvania, Department of Pathology.

CHARLES H. KELLAWAY,
Director.

The Walter and Eliza Hall Institute of Research in Pathology and Medicine

FINANCIAL STATEMENT FOR THE YEAR ENDED 30th JUNE, 1942.

INCOME ACCOUNT.

RECEIPTS.

To Balance 30/6/41				£5,280	6	9
,, Trustees, W. & E. Hall Trust	£3,200	0	0			
,, University of Melbourne	750	0	0			
,, Felton Bequests Committee	270	0	0			
,, Estate Anthony Mackie	150	0	0			
,, Estate Alfred Edments	100	0	0			
,, Royal Melbourne Hospital Refund	445	11	3			
,, Interest on Investments	141	2	7			
,, Army and Air Force—Blood Typing	2,438	18	9			
,, Fees Received for Tests	491	19	11			
				7,987	12	6
				£13,267	19	3

EXPENDITURE.

By Salaries and Wages	£4,185	14	2			
,, Materials	823	11	6			
,, Apparatus	216	3	8			
,, Printing, Stationery, Postages, etc.	218	0	3			
,, Pay Roll Tax	133	7	1			
,, Publications	656	15	1			
,, Fittings and Equipment	18	9	0			
,, Repairs to Apparatus	19	0	7			
,, Repairs to Buildings	0	7	7			
,, Biochemical Department	382	16	0			
,, Library	18	9	3			
,, Expenses—New Building	95	0	0			
				£6,767	14	2
,, Balance—						
Investments	3,947	13	2			
Cash—Agent-General, London	34	2	7			
E. S. & A. Bank	100	0	0			
Bank of N.S.W.	2,418	9	4			
				6,500	5	1
				£13,267	19	3

ELSIE MARION CARTY ACCOUNT.

To Balance 30/6/41		£310 18 4	By Salary	£250 0 0	
,, Estate E. M. Carty	£33 4 6		,, Materials	9 0 7	
,, Interest on Investments	175 19 5		,, Sundries	8 2 10	
		209 3 11			£267 3 5
			,, Balance		252 18 10
		£520 2 3			£520 2 3

VIRUS ACCOUNT.

To Balance 30/6/41		£822 12 5	By Salaries and Wages	£1,095 14 5	
,, Commonwealth Government	£1,529 6 10		,, Materials	1,315 5 11	
,, Mr. E. Alec Cato	1,000 0 0		,, Pay Roll Tax	20 18 3	
		2,529 6 10			2,431 18 7
			,, Balance		920 0 8
		£3,351 19 3			£3,351 19 3

ARMY MEDICAL RESEARCH FUND.

To Balance 30/6/41		£794 7 9	By Salary	£177 16 1	
			,, Materials	246 11 10	
			,, Sundries	10 0 0	
					£434 7 11
			,, Balance		359 19 10
		£794 7 9			£794 7 9

NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL GRANTS.

	£	s.	d.		£	s.	d.	£	s.	d.
To Balance 30/6/41	£254	2	7	By Salaries and Wages	£2,309	8	4			
,, Commonwealth Government	2,680	1	8	,, Materials	161	7	5			
				,, Virus Account	73	1	10			
				,, Travelling Expenses	15	11	6			
								2,559	9	1
				,, National Health and Medical Research Council—Refund of Unexpended Grants				374	15	2
	£2,934	4	3					£2,934	4	3

ENDOWMENT FUND.

	£	s.	d.		£	s.	d.
To Biochemical Endowment Fund	£9,088	12	9	By Investments	£22,149	3	10
,, Elsie Marion Carty Fund	4,531	3	6	,, Bank of N.S.W.	0	12	5
,, Library Fund	2,030	0	0				
,, G. C. M. Mathison Fund	6,500	0	0				
	£22,149	16	3		£22,149	16	3

AUDITOR'S CERTIFICATE.

I have to report that I have completed the Audit of the Books and Accounts of the Institute for the period ended 30th June, 1942. I have verified all receipts and have had vouchers produced for all disbursements. All information and explanations required have been given. The Statement is a correct statement of Receipts and Expenditure as revealed by the Books of the Institute.

3rd August, 1942.

W. M. JARVIE, F.C.A. (Aust.), Auditor.